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Mutations Conferring Resistance to Zidovudine Diminish the Antiviral Effect of Stavudine Plus Didanosine

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This study evaluated the influence of zidovudine (ZDV) resistance mutations on the antiviral effect of the combination of stavudine (D4T) plus didanosine (ddI) in patients treated previously with ZDV plus zalcitabine (ddC). Twenty patients who had been treated with ZDV plus ddC for a median duration of 11 months (range, 7–42 months) were switched to D4T (40 mg twice a day [BID]) + ddI (200 mg BID) in an open pilot study lasting 6 months. The CDC classes were A ($n = 10$) and B ($n = 10$). The median baseline CD4 count was 285/mm^3 and the median baseline plasma virus RNA (Amplicor HIV Monitor RT-PCR assay) was 4.6 log copies/ml. Population-based sequence analysis detected mutations associated with resistance to reverse transcriptase (RT) inhibitors in the RT domain of virus RNA from baseline plasma samples in 13/20 (65%) patients. Twelve patients had mutations associated with zidovudine resistance (3 T215Y - M41L - L210W; 3 T215Y - M41L; 2 T215Y - L210W; 3 T215Y; 1 K70R) and 1 patient had a multi-dideoxynucleoside resistance mutation (QI5IM). Patients with a resistance mutation had a significantly lower RNA suppression after 3 and 6 months (median RNA reduction –0.5 log and –0.1 log) than the remaining patients (–1.6 log and –2 log). Fifty percent of patients with wild-type viruses had undetectable plasma RNA after 24 weeks of D4T plus ddI therapy, whereas all those with mutated viruses had HIV RNA concentration > 3 log copies/ml at week 24 ($P < .05$). Our finding may have implications when deciding on a second line therapy with three or four drugs that includes two new nucleoside analogues. Cross-resistance between nucleoside analogues deserves maximal attention to ensure optimal antiretroviral therapy and design algorithms for antiretroviral management based on genotypic antiretroviral resistance testing. ***J. Med. Virol. 59:507–511, 1999.***

KEY WORDS: HIV resistance; zidovudine mutations; stavudine plus didanosine

INTRODUCTION

The treatment recommended currently for human immunodeficiency virus (HIV) disease is a triple-drug regimen consisting of two nucleoside inhibitors of reverse transcriptase (RT) and a protease inhibitor [Carpenter et al., 1998; Gazzard et al., 1998]. Both a thymidine analogue RT inhibitor and a non-thymidine analogue RT inhibitor are included in the triple-drug regimen because of their different activities in activated and resting cells. The combination of stavudine (D4T) and didanosine (ddI) as a potential backbone of multiple-drug therapy offers several advantages. These advantages include good penetration into peripheral compartments by D4T and reasonably good cerebrospinal fluid penetration by ddI, the rare development of resistance over time to both D4T and ddI, and activity of ddI in long-lived monocytes and macrophages.

The use of the combination of D4T and ddI to treat patients treated previously with zidovudine (ZDV) plus zalcitabine (ddC), has the advantage of using two new nucleoside analogues. Several studies have shown that the emergence of ZDV resistance mutations is not prevented by combination therapy with ZDV plus ddC, whereas mutations conferring resistance to ddC are detected rarely [Richman et al., 1994; Larder et al., 1996; Schooley et al., 1996; Brun-Vézinet et al., 1997]. Although the resistance mutation profiles of ZDV, D4T, and ddI are different [Schinazi et al., 1996], few data are available on the intrinsic antiviral effect of the D4T plus ddI combination in patients in whom ZDV- resis-

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tant viruses may have emerged. In a recent study, Raffi et al. [1998] have shown that treating HIV-infected patients treated previously with ZDV and/or ddC with D4T plus ddI combination therapy is safe and leads to a sustained antiviral effect, at least in some patients.

This study was begun before protease inhibitors became available and was designed to evaluate the efficacy of D4T plus ddI for treating HIV-infected patients treated previously with ZDV plus ddC. We have assessed the influence of mutations associated with ZDV resistance on the antiviral effect of D4T plus ddI.

MATERIALS AND METHODS

Design

This was an open-label pilot study conducted at the Department of Infectious Disease of Toulouse University Hospital, France. Eligible patients were given D4T plus ddI for 24 weeks. They all had documented HIV infections and were no longer benefitting virologically or immunologically from the first line therapy with AZT plus ddC given for at least 6 months. Virological and immunological failures were defined as a return to baseline values of HIV RNA and the CD4 cell count. Patients were older than 18 years of age and had hemoglobin concentrations of more than 80 g/L, absolute neutrophil counts of more than 0.6×10^9/L, platelet counts of more than 50×10^9/L, and no history of prior pancreatitis or peripheral neuropathy. All patients signed informed consent forms prior to enrolment.

Treatment Regimen

Patients weighing more than 60 kg were given 40 mg D4T and 200 mg ddI each twice daily; those weighing less than 60 kg were given 30 mg D4T and 125 mg ddI, each twice daily.

Follow-Up

The patients were evaluated every 4 weeks for signs and symptoms of HIV disease. Adverse events and concomitant medication were recorded. Blood was taken for haematology and chemistry, CD4 cell counts, and plasma HIV-1 RNA assay at baseline, week 4, week 12, and week 24. The RT domain of virus RNA from baseline plasma samples was sequenced.

Plasma HIV-1 RNA

The HIV-1 RNA in the plasma was measured using the Amplicor HIV-1 Monitor reverse transcription-polymerase chain reaction (RT- PCR) assay (Roche Diagnostic Systems, Neuilly, France) according to the manufacturer's instructions.

CD4 Lymphocyte Counts

Peripheral blood CD4 lymphocytes were counted by flow cytometry (Epics Profile; Coulter, Hialeah, FL) using commercially available monoclonal antibodies (Beckton Dickinson, Mountain View, CA).

Nucleotide Sequencing of the HIV-1 RT Gene

Both strands of a nested PCR product from the HIV-1 genome encoding the first 240 amino acids of the RT were sequenced directly. The outer primers were RT2 (antisense primer, 5′- TCTACTTGTCCATGCATGGCTTC-3′) and RT1 (sense primer, 5′-GGAAACCAAAAATGATAGGGGGAATTGGAGG-3′); the inner primers were RT4 (antisense primer, 5′-ATGTCATTGACAGTCCAGCT-3′) and RT3 (sense primer 5′-ATTTTCCCATTAGTCCTATT-3′). RNA was extracted from 100 μl plasma by the guanidinium thiocyanate-phenol-chloroform method. Reverse transcription was carried out with 20 units M-MuLV reverse transcriptase (Boehringer-Mannheim, Mannheim, Germany) and the primary antisense primer. PCR was carried out in 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleoside triphosphate, 50 pmol of each primer, 2.5 units *Taq* polymerase (AmpliTaq, Perkin Elmer Cetus, Norwalk, CT), and 5 μl cDNA solution. The primary PCR involved initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 60 sec, annealing at 55°C for 60 sec, and polymerisation at 72°C for 150 sec with a final elongation at 72°C for 10 min. An aliquot (5 μl) of the primary PCR products was used for 35 cycles of nested PCR as follows: initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 sec, annealing at 54°C for 30 sec, and polymerisation at 72°C for 60 sec with a final elongation at 72°C for 5 min. The RT3-RT4 amplification gave an 800-bp product, which was purified using QIAamp columns (Qiagen, Courtaboeuf, France) and sequenced on both strands by the dideoxy chain termination method (ABI PRISM Ready Reaction AmpliTaq Fs, Dye Deoxy Terminators, Applied Biosystems, Paris, France) on an ABI377 automated DNA sequencer (Applied Biosystems). All the measures to prevent contamination suggested by Kwok and Higushi [1989] were applied strictly.

Statistical Analysis

HIV-1 RNA concentrations were transformed to $\log_{10}$ values before analysis. Specimens in which HIV-1 RNA was undetectable were assigned the value of the detection limit of the Monitor assay (i.e., 2.3 log copies/ml). Quantitative variables were analysed by the Mann–Whitney test. Qualitative variables were analysed by the chi-squared test or Fisher's exact probability test. A value of $P < .05$ was considered to be statistically significant.

RESULTS

Patient Characteristics at Baseline

A group of 20 HIV-1 infected patients (16 men and 4 women; mean age, 36 years) who had been treated with ZDV plus ddC were enrolled between December 1995 and October 1996. Ten patients were group A and 10 were group B (Centers for Disease Control and Prevention 1993 criteria) at the time of enrolment. The median baseline CD4 cell count was 285/mm^3 (range, 170–

TABLE I. Amino Acid sequences of Baseline Isolates From Patients in Whom Mutations Associated With Zidovudine and Multi-Dideoxynucleoside Resistance Were Detected

	Plasma HIV RNA (log copies/ml)		CD4 cell count (/mm^3)		Zidovudine resistance mutations at baseline						Multidideoxynucleoside resistance mutations at baseline				
Patient	Baseline	Week 24	Baseline	Week 24	41 M	67 D	70 K	210 L	215 T	219 K	62 A	75 V	77 F	116 F	151 Q
1	4.8	ND	242	ND	L	—	—	W	Y	—	—	—	—	—	—
2	3.3	−0.1	260	+109	L	—	—	W	Y	—	—	—	—	—	—
3	4.6	ND	418	ND	L	—	—	W	Y	—	—	—	—	—	—
4	2.3	+1.5	255	−21	L	—	—	—	Y	—	—	—	—	—	—
5	3.9	−0.1	424	+98	L	—	—	—	Y	—	—	—	—	—	—
6	5.7	−0.4	196	+565	L	—	—	—	Y	—	—	—	—	—	—
7	6.3	ND	115	ND	—	—	—	W	Y	—	—	—	—	—	—
8	4.9	−0.1	254	+215	—	—	—	W	Y	—	—	—	—	—	—
9	4.9	+0.4	330	−5	—	—	—	—	Y	—	—	—	—	—	—
10	4.9	−1.7	220	+27	—	—	—	—	Y	—	—	—	—	—	—
11	4.7	−0.4	302	+84	—	—	—	—	Y	—	—	—	—	—	—
12	4.4	+0.5	170	+77	—	—	R	—	—	—	—	—	—	—	—
13	2.8	ND	207	ND	—	—	—	—	—	—	—	—	—	—	M

ND, not determined. Substitutions at codons associated with zidovudine resistance (codons 41, 67, 70, 210, 215 and 219) and multidideoxynucleoside resistance (codons 62, 75, 77, 116 and 151) are shown. Dashes indicate identity with the consensus Clade B sequence shown in the top row. Abbreviations for amino acids: A, Ala; D, Asp; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; T, Thr; V, Val; and Y, Tyr.

424) and the median baseline plasma virus RNA was 4.6 log copies/ml (range, 2.3–6.3). The median duration of prior ZDV plus ddC therapy was 11 months (range, 7–42).

Mutations associated with resistance to RT inhibitors were detected in 13/20 (65%) patients. Of these, 12 had mutations associated with ZDV resistance (3 T215Y - M41L - L210W; 3 T215Y - M41L; 2 T215Y - L210W; 3 T215Y; 1 K70R) and 1 had a multi- dideoxynucleoside resistance mutation (Q151M) (Table I). The presence of resistance-associated mutations was not related to the duration of ZDV plus ddC therapy, clinical stage, CD4 cell count or plasma RNA at baseline.

HIV RNA and CD4 Cell Count Responses

Fifteen of the 20 patients completed the 24-week course of D4T plus ddI therapy. Of the other 5 patients, 1 withdrew at month 2 because of side effects (gastrointestinal intolerance and psychiatric problems) and 4 because of virological failure after 3 months of therapy, i.e., RNA reduction from baseline of less than 0.5 log copies/ml. There was a median gain in CD4 cell count of 58/mm^3 and a median decrease of 0.7 log copies/ml in virus plasma RNA at week 12 (Table II). The median gain in CD4 cell count was + 88/mm^3 and the median decrease in plasma virus RNA was −0.4 log copies/ml at week 24. The plasma RNA concentrations in 3/15 patients (20%) at week 24 were below the detection limit of the Monitor RT-PCR assay.

Influence of Resistance-Associated Mutations on HIV RNA and CD4 Cell Count Responses

As shown in Figure 1, patients harboring viruses with resistance mutations in the RT gene had a significantly less RNA suppression after 3 and 6 months (median RNA reduction −0.5 log copies/ml and −0.1 log copies/ml) than the patients with wild-type viruses (median RNA reduction −1.6 log copies/ml and −2 log copies/ml) ($P < .05$ at month 3, $P < .01$ at month 6). These differences in viral RNA response to the D4T + ddI treatment were differences between the patients with and without baseline resistance toward AZT because the only patient carrying the Q151M mutation withdrew at month 2. Of the 6 evaluable patients with wild-type viruses, 3 (50%) had undetectable plasma RNA after 24 weeks of D4T plus ddI therapy. By contrast, 9/9 (100%) evaluable patients with mutated viruses had HIV RNA concentration more than 3 log copies/ml at week 24 ($P < .05$). A rebound toward baseline in HIV RNA concentrations occurred in all but one patients with mutated viruses.

The median increases in CD4 cell count in patients who had mutated viruses (+34/mm^3 at week 12 and +84/mm^3 at week 24) were smaller than those in patients with wild-type viruses (+74/mm^3 at week 12 and +98/mm^3 at week 24), but the difference was not statistically significant.

DISCUSSION

Because the aim of antiretroviral therapy is to obtain maximal suppression of virus replication to reduce the emergence of drug resistance, it is critical to determine the intrinsic antiviral effect of nucleoside combinations used as the foundation of triple or quadruple therapy regimens. This study shows that the virological response of patients treated previously with ZDV plus ddC to D4T plus ddI depends on the presence or absence of ZDV resistance mutations. Only patients harboring wild-type viruses had sustained reductions of HIV-1 RNA after 24 weeks of therapy.

Although the overall HIV RNA and CD4 cell responses showed good efficacy after 24 weeks of D4T plus ddI therapy in patients treated previously with ZDV plus ddC, i.e., a mean decrease of 0.9 log RNA copies/ml and an increase in the mean CD4 cell count of 112/mm^3, not all patients responded in the same way to this combination therapy. This non-uniform response of ZDV- experienced patients to the D4T plus ddI com-

TABLE II. Change From Baseline in HIV Plasma RNA and CD4 Cell Count

	Baseline	Week 4	Week 12	Week 24
Plasma HIV RNA (log copies/ml)				
No. patients	20	20	19	15
Mean	4.6	−0.8	−0.8	−0.9
Median	4.7	−0.6	−0.7	−0.4
Range	2.3–6.3	+1.5 to −2.6	+1 to −1.7	+1.5 to −3.3
CD4 cell count (/mm^3)				
No. patients	20	20	19	15
Mean	266	+80	+93	+112
Median	258	+78	+58	+88
Range	115–424	−112 to +308	−103 to +573	−21 to +565

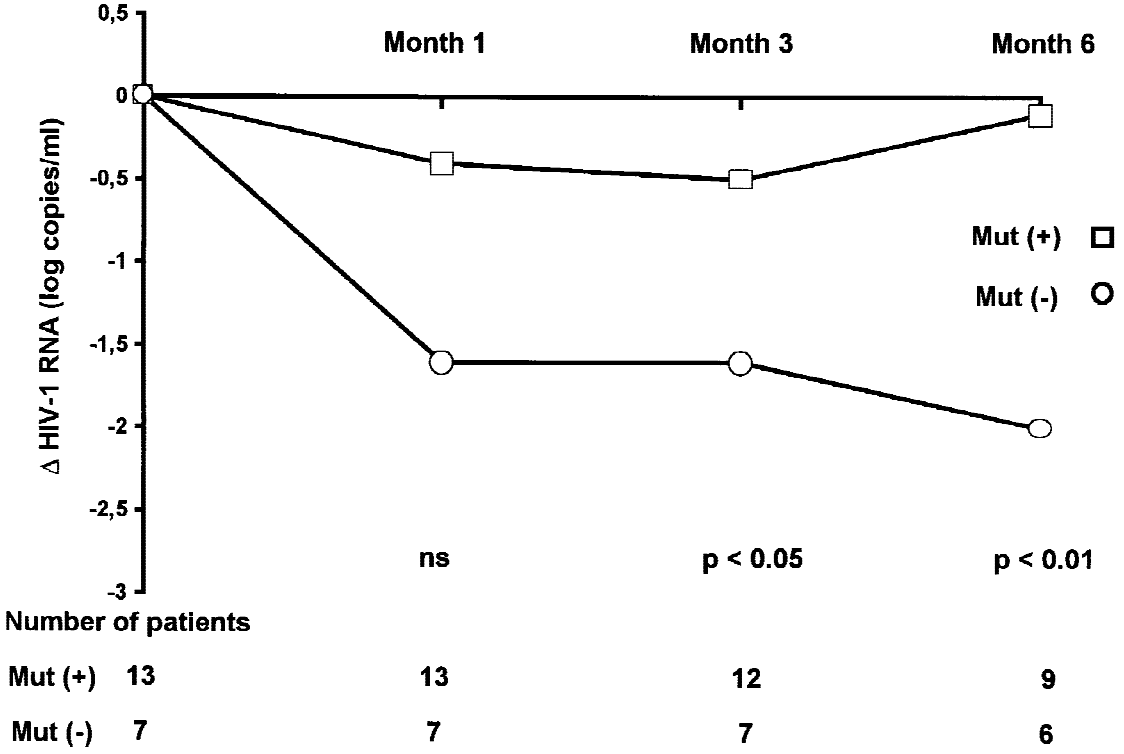

Fig. 1. Change in median concentration of plasma human immunodeficiency virus-1 (HIV-1) during the 6-month course of stavudine (D4T) plus didanosine (ddI) in patients with resistance mutations in the reverse transcriptase (RT) gene at baseline (Mut +, □) and in those without resistance mutations (Mut −, ○).

bination was also described in a study that suggested that this response could be due to different levels in intracellular phosphorylation of D4T [Raffi et al., 1998]. Nevertheless, the part played by altered phosphorylation due to down-regulation of intracellular kinase enzymes in the failure of nucleoside RT inhibitors is controversial. In a recent study, there was no significant difference in intracellular D4T triphosphate levels and the ratio of D4T triphosphate to thymidine triphosphate between ZDV-naïve and ZDV-experienced individuals [Phiboonbanakit et al., 1999].

Although no clear cross-resistance patterns between ZDV and D4T or ddI have been identified from in vitro or in vivo studies, we assessed the influence of ZDV resistance mutations on the virological response to D4T plus ddI. The HIV RNA concentration of the patients harboring viruses with mutations associated with ZDV resistance rebounded to initial levels after 24 weeks therapy following a very limited initial drop. In contrast, the drop in the virus RNA concentration in the patients harboring wild-type virus during the 24 weeks was very similar to that observed in naïve patients treated with the same D4T plus ddI combination, with a reduction of about 2 log copies/ml after 24 weeks [Segondy et al., 1998]. Two factors may explain the lower efficacy of D4T plus ddI in patients harboring ZDV resistance mutations. First, the level of HIV RNA in plasma that contained a codon 215 mutant genotype is predictive of a poor virological response after addition of ddI to ZDV-experienced subjects [Holodniy et al., 1995]. In addition, the ACTG 116 B/117 protocol showed that a high phenotypic resistance to ZDV before treatment with ddI (50% inhibitory ZDV concentration > 1 μM) is associated with an increased risk of

clinical progression [D'Aquila et al., 1995; Japour et al., 1995]. Second, recent in vitro susceptibility studies have shown a correlation between phenotypic resistance to ZDV and phenotypic resistance to D4T [Descamps et al., 1998]. Moreover, mutations associated with ZDV resistance including the T215Y/F mutation have been described in antiretroviral-naïve patients receiving D4T and ddI in combination [Coakley et al., 1999]. These data suggest a cross-resistance between ZDV and D4T.

In summary, whether or not the antiviral effect of D4T plus ddI in patients treated previously with ZDV plus ddC is satisfactory seems to depend on the presence or absence of ZDV- resistance mutations. This finding may have clinical implications when deciding on the optimal combination of nucleoside RT inhibitors as a component of three or four drug regimens. This finding may have also implications for designing algorithms for antiretroviral management based on genotypic antiretroviral resistance testing.

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